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Polymorphism in the *Helicobacter pylori* CagA and VacA toxins and disease

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Half of the world's population is infected with *Helicobacter pylori* and approximately 20% of infected individuals develop overt clinical disease such as ulcers and stomach cancer. Paradoxically, despite its classification as a class I carcinogen, *H. pylori* has been shown to be protective against development of asthma, allergy, and esophageal disease. Given these conflicting roles for *H. pylori*, researchers are attempting to define the environmental, host, and pathogen interactions that ultimately result in severe disease in some individuals. From the bacterial perspective, the toxins, CagA and VacA, have each been shown to be polymorphic and to contribute to disease in an allele-dependent manner. Based on the notable advances that have recently been made in the CagA field, herein we review recent studies that have begun to shed light on the role of CagA polymorphism in *H. pylori* disease. Moreover, we discuss the potential interaction of CagA and VacA as a mediator of gastric disease.

Introduction

In 1982, the Gram-negative, spiral shaped microaerophilic bacterium *Helicobacter pylori* was first isolated from the stomach of a patient with gastritis.[1] Thirty years later, *H. pylori* has been shown to colonize over half of the world's population. In approximately 20% of infected individuals, infection with *H. pylori* may result in clinical disease that manifests as peptic or duodenal ulcers, gastric adenocarcinoma, or mucosa-associated lymphoid tissue (MALT) lymphoma;[2-5] these last two manifestations have led *H. pylori* to be the only bacterium classified as a class I carcinogen by the World Health Organization.[6] For the remaining 80% of infected individuals, the chronic inflammatory response induced by *H. pylori* in the gastric mucosa results in no overt clinical symptoms.

H. pylori, is the predominant bacterial species known to colonize the stomach, and the bacterium is typically acquired early in childhood; in the absence of antibiotic treatment, lifetime colonization occurs.[7] *H. pylori* association with humans is hypothesized to have originated in Africa preceding the human migration that is believed to have occurred approximately 58,000 years ago.[8,9] As humans migrated from Africa to various continents, *H. pylori* was carried with them and evolved into present day strains; this close association has been used to study human migration patterns.[10-14] Due to the large number of colonized individuals, the majority of which do not develop disease, and because *H. pylori* has evolved with humans for centuries, there is currently a movement to classify *H. pylori* as normal human flora (discussed below).

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H. pylori Classification and Role in Health and Disease

Since its discovery, *H. pylori* has been shown to be one of the most variable species of bacteria. This heterogeneity has been the basis for a series of classification systems that have been used to discriminate between strains. Initially, *H. pylori* strains were categorized as type I or type II based on the presence or absence of both the cytotoxin-associated gene-pathogenicity island (cag-PAI) and the vacuolating cytotoxin, VacA, respectively.[15-17] Following the identification of allelic variation in *cagA*, which is carried on the island and encodes for the CagA toxin, strains were next classified as either East Asian or Western based on the particular *cagA* allele that they carried. This system specifically utilizes variation in the C-terminus of the protein to distinguish strains. East Asian strains are predominantly from Japan, South Korea, and China, while Western stains are primarily from the United States, Europe, and Australia.[18-20] Over the last several years, the discovery of additional *cagA* alleles led to the addition of the J-Western and Amerindian strain designations. The J-Western, or Japanese subtype of Western CagA, strain was initially believed to be predominantly found in Okinawa, Japan;[21,22] however, it has recently been shown that J-Western strains have a broader geographic distribution.[23] The Amerindian strain is predominately found within the Amerindian populations of South America.[10,24,25] Finally, multilocus sequence typing (MLST) of several *H. pylori* housekeeping genes and the *vacA* virulence gene has been utilized to develop a classification system that takes advantage of the broader genetic diversity of *H. pylori* as a means to study its ancestry;[9,12,13,26] MLST identified seven distinct *H. pylori* populations, some of which have several sub-populations that are named based upon the geographic location in which the strains are found (**Table 1**).[9,13,14,26-28] This new classification system delineates strains from the classical East Asian, Western, Amerindian or J-Western strain designations, which are based solely on *cagA* C-terminal polymorphisms.

In keeping with the idea that *H. pylori* may be normal flora whose association with the human host is important, recent

Table 1. An overview of *H. pylori* populations*

H. pylori population	*H. pylori* subpopulations	Geographic location or human population	Reference
hpAfrica1	hspWAfrica	W. Africa	13
	hspSAfrica	S. Africa	13
hpAfrica2		S. Africa	13
hpNEAfrica		Ethiopia, N. Nigeria, Somalia, Sudan	9
hpAsia2		Bangladesh, N. India, Malaysia, Thailand	9, 28
hpEastAsia	hspAmerind	Native Americans	13
	hspEAsia	East Asians	13
	hspMasori	Taiwanese Aboriginals, Melanesians, Polynesians	14
hpEurope		Europe, Middle East, India, Iran	13, 27
hpSahul		Australian Aboriginals, Papua New Guineans	14

*Table 1 was modified from reference 26.

Table 2. Role of *H. pylori* as normal flora and pathogen^

Potential beneficial effects of *H. pylori* against:	Known and suspected negative effects of *H. pylori:*
Asthma[29-32,183,184]	Gastritis[1,185]
Allergy[31,184]	Peptic and duodenal ulcers[185,186]
Esophageal reflux and Barrett esophagus[41,42,45]	MALT lymphoma[2]
Esophageal adenocarcinoma[39,40]	Gastric adenocarcinoma[2,4,5]
Reactivation of tuberculosis*[34,35]	Idiopathic thrombocytic purpura (reviewed in ref. 37)
Inflammatory bowel diseases*[187,188]	Iron deficiency anemia (reviewed in ref. 37)
Weight gain (due to effects on leptin and ghrelin)*[36,38]	Atherosclerosis/coronary artery disease*[189,190]
Graves disease*[191]	

*Further analysis is required to substantiate these findings. ^Due to space limitations only a few key references are provided.

findings suggest that *H. pylori* plays a protective role in various aspects of human health (**Table 2**). Perhaps the strongest evidence for a protective role for *H. pylori* against disease development are the studies that show an inverse association between *H. pylori* colonization with occurrence of asthma and allergy, particularly in adolescents.[29-32] Interestingly, the presence of CagA seems to increase the protective nature of *H. pylori* against both asthma and allergy.[31] Therefore, the disappearance or eradication of *H. pylori* may represent another example of the "Hygiene Hypothesis," which suggests that reduced exposure to pathogens early in life may predispose individuals to diseases such as allergy and asthma later in life.[33] A protective role for *H. pylori* that extends beyond the "Hygiene Hypothesis" has been indicated by studies that show that *H. pylori* protects against tuberculosis and diabetes. The tuberculosis study utilized human subjects as well as non-human primates to show that *H. pylori* colonized individuals are less likely to develop active tuberculosis through reactivation.[34] Additionally, *H. pylori* infected individuals have significantly increased Th1 responses and IFN-γ, which is required for TB latency.[35] In relation to diabetes, recent work indicates that the human gastric hormones that control energy homeostasis, leptin and ghrenlin, along with body mass index (BMI) are increased in patients following *H. pylori* eradication.[36,37] Furthermore, a cross-sectional study identified a positive association between glycated hemoglobin, a marker for long-term blood glucose levels, and *H. pylori* in individuals without diabetes; this association was increased in patients with a higher BMI.[38] Therefore, data suggest that eradication of *H. pylori* may play a role in development of metabolic disorders in individuals with an increased BMI.

Finally, as the prevalence of *H. pylori* has decreased over the last century, a rise in esophageal reflux disease and esophageal adenocarcinoma has been observed. Several studies suggest a protective role for *H. pylori* in preventing both diseases.[39-44] A meta-analysis of 49 studies, as well as two prospective studies, indicate that *H. pylori* colonization is associated with a reduced risk for Barrett esophagus, which often occurs following esophageal exposure to acid through reflux.[43-45] It is believed that the decrease in stomach acid, which occurs due to the loss of parietal cells in *H. pylori* infected individuals, protects the esophagus from cellular damage that initiates esophageal disease.[45] A potential role for CagA in protection against esophageal disease has been suggested by studies that indicate individuals infected with *cagA* positive *H. pylori* strains are less likely to develop esophagitis or Barrett esophagus than those infected with *cagA* negative strains.[42,43] These results in turn suggest that there may be a consequence to outright eradication of *H. pylori* from the human microbiome.

Given the clear evidence that *H. pylori* is associated with development of gastric disease, along with the growing evidence that

Table 3. An overview of factors influencing *H. pylori* disease development

	Factor or mechanism	References^
Host:	Age	175, 192
	Male sex	148, 190
	IL-1 gene cluster polymorphisms	193–195
	IL-10 gene polymorphisms	195, 196
	HLA gene haplotype	197
	TLR2 and TLR4 gene types	198
	Atg16L1 Autophagy gene polymorphism	199
	Nod1/Nod2 polymorphism (Chinese population)	200
	PTPN11 gene polymorphism	201, 202
	Gastric mucins	203
Bacterial:	*cag* pathogenicity island	15
	CagA EPIYA polymorphism	109–113
	vacA polymorphism	138–140, 142, 144, 147
	Outer membrane proteins (HomB, BabA, SabA/B, IceA, OipA, DupA)	175
Environmental:	Diet: Red meat/processed meat consumption and production of nitrosamines*	63
	Asian Populations: High salt consumption*	204
	Mexican Populations: Capsaicin consumption	61
	Antibiotic use	205
	Smoking:	204, 206, 207
	CagA positive *H. pylori*	208
	Geographical location: East Asia vs. North America/Europe/Africa vs. South America:	10, 18–22, 24, 25

*Association with disease was found only in individuals with concurrent *H. pylori* infection. ^Due to space limitations, only a few key references are provided.

suggests that *H. pylori* may play a beneficial role in some individuals, the question arises as to what factor/factors are responsible for determining the ultimate outcome of *H. pylori* colonization? Unfortunately, there is likely no single answer to this question. Instead, as previously stated by Merrell and Falkow, "the balance shift that causes colonization to go awry, leading to a deadly disease, is a combination of physiological and genetic factors for both participants of the host-pathogen interaction."[46] Clearly, in the case of *H. pylori*-induced gastric disease, the etiology involves numerous host genetics/responses, environmental factors, and bacterial virulence factors (**Table 3**). Accordingly, previous reviews have focused on these topics.[47,48] As such, herein, we will only briefly mention some discoveries that affect our understanding of host and environmental factors, and then focus the remainder of our discussion on bacterial factors that are clearly important for disease.

Host and Environment

From the immunological perspective, it is known that how the immune system responds to *H. pylori* dictates the ultimate outcome of infection and that the immune response to *H. pylori* is clearly complicated (refer to reviews in refs. 49–51). For example, specific classes of T-cells have several possible effects. The presence of T-cells promotes *H. pylori*-induced chronic-active gastritis.[52] As such, patients with peptic ulcer disease show an increase in Th1 and Th2 cellular responses, which likely then contribute to the pathology associated with gastric disease.[53] In contrast, the role of T-regulatory (Treg) cells in *H. pylori* infection appears protective (reviewed in ref. 49). Tregs control effector T-cell responses to *H. pylori* infection in a neonatal mouse model by inducing tolerance and protecting against development of preneoplastic lesions.[54] Furthermore, an increased number of $CD4^{+}CD25^{hi}$ Treg cells that secrete the immune suppressive cytokine IL-10 is inversely correlated with IL-8 expression and NF-κB activation in asymptomatic carriers.[53] In keeping with this, mild gastritis observed in *H. pylori* infected children is also linked to a Treg cell response along with increased levels of IL-10 and TGF-β.[55] Since *H. pylori* is known to induce IL-8 expression upon host cell contact, which in turn induces proinflammatory conditions in the stomach,[56,57] it is possible that the presence of a Treg cell response regulates IL-8 expression and NF-κB activation by suppression of the proinflammatory cytokine Il-17 from the gastric mucosa as a means to prevent overt disease and bacterial clearance (refer to review in ref. 51). This notion is consistent with the finding that the presence of a Treg cell population results in increased *H. pylori* colonization.[53]

Supporting the idea that specific T-cell immune responses determine the outcome of *H. pylori* infection, recent studies indicate that Th17 cells promote *H. pylori* associated pathology. In

the absence of the Th17 polarizing cytokine IL-23, IL-23p19$^{-/-}$ mice exhibit significantly less gastritis and precancerous lesions, while IL-23$^{-/-}$ *H. pylori* infected mice show decreased gastritis, decreases in the cytokines IL-17 and IFN-γ, and concomitant increases in bacterial burden.[58,59] IL-23 therefore appears to contribute to gastric pathology through activation of Th1/Th17 immune responses that control *H. pylori* infection by initiating inflammation and controlling the degree of gastritis.[58,59] The results from these animal experiments have also been extrapolated to human samples. Analysis of patients with a past, but not current, *H. pylori* infection shows persistent *H. pylori* specific Th17 responses. Among these individuals, those with gastric pre-cancerous lesions have increased levels of IL-17A compared with those without lesions.[60] In summary, the type of immune response mounted by the host influences the outcome of *H. pylori* infection.

From the environmental perspective, diet and geographical location appear to have the largest influence on disease. Several studies have indicated that consumption of nitrosamines in processed meats and vegetables, as well as a high salt diet both increase the risk for *H. pylori* induced disease.[61-64] Due to variations in diet around the world, the role of diet in the incidence of gastric carcinoma also varies. In East Asia, where the incidence of gastric carcinoma is high, diets are often high in salt content and nitrosamines (reviewed in ref. 64 and discussed in ref. 63). Similarly, in Europe, consumption of nitrosamines is associated with gastric carcinoma.[63] Studies of Mexican populations, find that consumption of capsaicin, a component of chili peppers, may increase the risk of gastric carcinoma, particularly in individuals who carry an IL1B-31C allele.[61] While the role of nitrosamines, and other dietary components, have been studied in human populations, direct causation between diet, *H. pylori*, and disease is difficult to prove due to the inability to directly regulate patient's diets. As such, researchers are now using long-term colonization of dietary controlled non-human primates to address these questions. Indeed, gastric neoplasia is induced in rhesus macaques only when nitrosamine compounds are administered in the presence of *H. pylori* colonization; thus supporting the synergistic role of these compounds in gastric disease.[63]

Overall, the data suggest that as humans and *H. pylori* have co-evolved over the last 58,000 y, *H. pylori* has developed mechanisms to survive the harsh conditions found in the gastric environment, as well as mechanisms by which to evade and utilize the human immune response for survival. Conversely, humans appear to utilize *H. pylori* colonization as a means to protect against several severe disease states. However, despite the evidence that supports the protective role and generally asymptomatic colonization of *H. pylori* in the vast majority of individuals, some individuals are clearly predisposed to severe disease as a result of colonization. Thus far, host polymorphisms, bacterial polymorphisms, and environmental conditions (**Table 3**) have all been implicated in conversion of *H. pylori* from a commensal to a pathogen, but the precise mechanism underlying this conversion remains unclear. What is clear is that overall disease severity is attributed to the production of several *H. pylori* virulence factors, each of which show variability across strains: such as the toxins, CagA and VacA, and the outer membrane proteins, HomB, BabA, IceA, SabA/B, DupA, and OipA. The remainder of this review will focus on the role of CagA polymorphism in cancer development, as it is the most severe disease pathology associated with *H. pylori* infection, as well as how polymorphism in VacA may contribute to disease.

CagA

CagA is one of the best-studied virulence factors of *H. pylori*; this toxin is 120–145 kDa in size, and is encoded by *cagA*, which is carried on the *cag*-PAI.[15] *H. pylori* strains that carry the PAI are known to be more virulent than those that do not.[15] Following translocation into the host cell by the type IV secretion system (T4SS), which is encoded for by the other genes contained within the PAI, CagA exerts its effects directly on eukaryotic cells.[16,65,66]

The exact role of CagA appears to be multifactorial. For instance, effective *H. pylori* colonization of the apical surface of polarized monolayers requires CagA mediated acquisition of nutrients.[67,68] CagA also regulates the host's immune response to *H. pylori* as a means to aid in persistence of the bacteria. In the presence of CagA, bone marrow derived dendritic cells (BMDC) from wild type C57BL/6 mice are unable to mature and clear *H. pylori*.[69] Furthermore, in BMDC's infected with wild type *H. pylori,* mRNA expression of the proinflammatory cytokines TNF-α and IL-12p40 are decreased, while mRNA expression of IL-10 is increased; this response is CagA dependent.[69] In CagA-transgenic mice (CagA-Tg), expression of CagA inhibits the ability of BMDCs to mediate the differentiation of CD4$^+$ T-cells to Th1 cells and the ability to respond to LPS.[69] From these studies, CagA appears to negatively regulate dendritic cells as a means to promote persistence of *H. pylori*.[69]

Following translocation into eukaryotic cells by the T4SS, CagA binds to phosphatidylserine in the plasma membrane of host cells and is phosphorylated on tyrosine residues found within conserved C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs;[70,71] the host cell kinases c-Src and c-Abl, are responsible for phosphorylation.[72-75] Phospho-CagA forms a complex with SHP-2, a protein tyrosine phosphatase.[16,66,76] Formation of this complex results in recruitment to, and constitutive activation of, SHP-2 at the host cell membrane.[71] The many downstream signaling events resulting from CagA-induced SHP-2 activation have been extensively reviewed elsewhere.[77-79] However, for the purpose of our discussion, we point out that the deregulation of SHP-2 by CagA results in cell scattering and elongation. This CagA induced cell elongation is termed the "hummingbird phenotype" due to the long extensions observed on infected cells and depends on Ras independent modification of Erk as well as the interaction of CagA with partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK).[16,80-82] The interaction of CagA with PAR1 is also important for stabilizing CagA within the eukaryotic cell following translocation; deletion of the C-terminal EPIYA motifs results in a significant decrease in the half-life of CagA.[83] Not only can CagA induce cell scattering and elongation in a phosphorylation-dependent manner, CagA can also initiate this process in a phosphorylation independent

manner through mediation of NF-κB and β-catenin activity.[84] Regardless of the mechanism underlying initiation of cell scattering and elongation, following CagA injection, changes in expression of genes involved in epithelial-mesenchymal transition, a process known to be involved in the promotion of cancer dissemination in metastatic cancers and tissue fibrosis, occurs.[85] As such, during *H. pylori* infection of AGS cells, epithelial cell markers are found to be downregulated while markers of mesenchymal cells are upregulated.[85]

CagA domains. Recent studies of CagA have investigated how the N- and C-terminal domains of the protein are involved in targeting CagA within the eukaryotic cell as well as modulating changes in host cell signaling. Studies using MDCK cells expressing various domains of CagA found that the N-terminus of the protein targets CagA to the cell-cell junctions and promotes cell migration. Conversely, the C-terminus targets CagA to the cytoplasm and is required for pseudopodial formation.[86] Conflictingly, independent studies using AGS cells and transfection of various CagA EPIYA constructs indicate that the C-terminal EPIYA motif is required for CagA membrane localization and that this occurs independent of phosphorylation.[87] A third study, which also used transfection of CagA but in MDCK cells, aimed to clarify the role of the N- and C-termini in CagA membrane localization. This work showed that CagA contains two independent domains that target CagA to the membrane; one at the N-terminus and one at the C-terminus.[88] Thus, the conflicting results of the two previous studies were due to the particular region found in the truncated CagA products, which were different in each study.[88] Based on these results, Steininger, et al.[89] have suggested the following model: the first 200 amino acids of CagA may serve as a membrane binding domain that is required to mediate proper CagA translocation into the host cell, targets CagA to cell-cell adhesions, and serves to inhibit EPIYA-mediated cell signaling. Conversely, a second membrane targeting domain found in the C-terminus functions to tether CagA to the plasma membrane for EPIYA-mediated signaling events; both the N- and C-terminal regions of CagA interact with each other to accomplish membrane tethering.[88,89] Interestingly, the N-terminal targeting domain appears to inhibit apical surface constriction and host cell elongation by reducing CagA effects on β-catenin, which is believed to be an important component that contributes to carcinogenesis.[88] Consequently, activity of the N-terminal domain on β-catenin signaling may contribute to inhibition of carcinogenesis.[88] It is worth noting that since these studies used truncated versions of CagA, it is unknown precisely how the domains of intact CagA interact inside the host cell.

More recently, another study has further fine-tuned our understanding of the different CagA domains.[90] Examination of CagA by X-ray crystallography shows an ordered N-terminus that is comprised of three domains (I, II, and III) (**Fig. 1A**); Domain I corresponds to residues 24–221, which overlaps with residues 1–200 in the previously described model.[88,90] Basic residues located in Domain II are responsible for CagA's interaction with phosphatidylserine located within the host cell membrane (**Fig. 1B**). Domain III was shown to contain a N-terminal binding sequence (NBS) that interacts with a C-terminal binding sequence (CBS) (**Fig. 1C**).[90] Consistent with a previous study that showed that CagA lacks a defined secondary structure,[91] NMR and X-ray crystallography revealed that tertiary structure of the C-terminus also lacks an ordered and solid structure.[90] It has been suggested that during interactions between the NBS and CBS, the NBS serves as a regulatory sequence that facilitates binding of CagA to its target proteins (**Fig. 1C**).[90] In this regard, interaction between the NBS and CBS may alter the C-terminal secondary structure whereby folding surrounding or within the Cag multimerization (CM) sequence may decrease turnover of the complex between CagA and PAR1/MARK; thus facilitating binding of other proteins.[90,92]

As alluded to in the previous paragraph, another component of CagA is a conserved 16 amino acid motif, FPL XRX XVX DLS KVG, found within the C-terminus. This motif mediates CagA multimerization and CagA's interaction with SHP-2; this segment is termed the Cag multimerization or CM sequence.[93,94] Furthermore, the presence of the 14 amino acid sequence, FPL KRH DKV DDL SK, within this region led to identification of this region as the MARK2 inhibitory sequence (MKI) due to its role as the binding site for PAR1/MARK kinase, which functions in CagA-mediated disruption of epithelial cell polarity.[82,91] The C-termini of both East Asian and Western *H. pylori* isolates contain CM sequences; in East Asian strains, this sequence is found immediately downstream of the EPIYA-D motif, and in Western strains this sequence is found upstream of each EPIYA-C motif and downstream of the last EPIYA-C motif (**Fig. 2A and B**).[94] This same region of the C-terminus has also been termed the conserved repeat responsible for phosphorylation independent activity (CRPIA) due to its role in promoting CagA phosphorylation independent activation of β-catenin and NF-κB signaling.[84]

En masse, these studies show that the various CagA domains contribute to targeting within the host cell, which ultimately affects how CagA alters host cell signaling pathways. While all of these CagA domain studies are intriguing, interpretation of the data are complicated due to use of transfection models, differences in the cell types used, and differences in the polarization state of the various cell lines.[86-88] Differences also occur between normal and cancer cell lines as well as between polarized epithelial monolayers and sub-confluent cells in terms of signaling and junctional complex formation.[95-97] Thus, it will be interesting to see if these results can be recapitulated in an infection model, especially since the levels of CagA are clearly different between these model systems.[77,89] One way to address these differences in model systems would be the creation of isogenic strains that could be used in a rodent model. Use of these same strains could then be expanded into a primate model as it is the closest model to replicate human disease.

CagA EPIYA motifs. While there has been some previous debate as to the role of the various domains of CagA, effects on host cell signaling are primarily attributed to the EPIYA motif region that is contained within the C-terminus of the protein (reviewed in ref. 77). The EPIYA motifs are comprised of Glu-Pro-Ile-Tyr-Ala residues, where the Tyr residues serve as the site of phosphorylation.[70] Differences exist in the number of EPIYA repeats found across strains, as well as in the amino acid

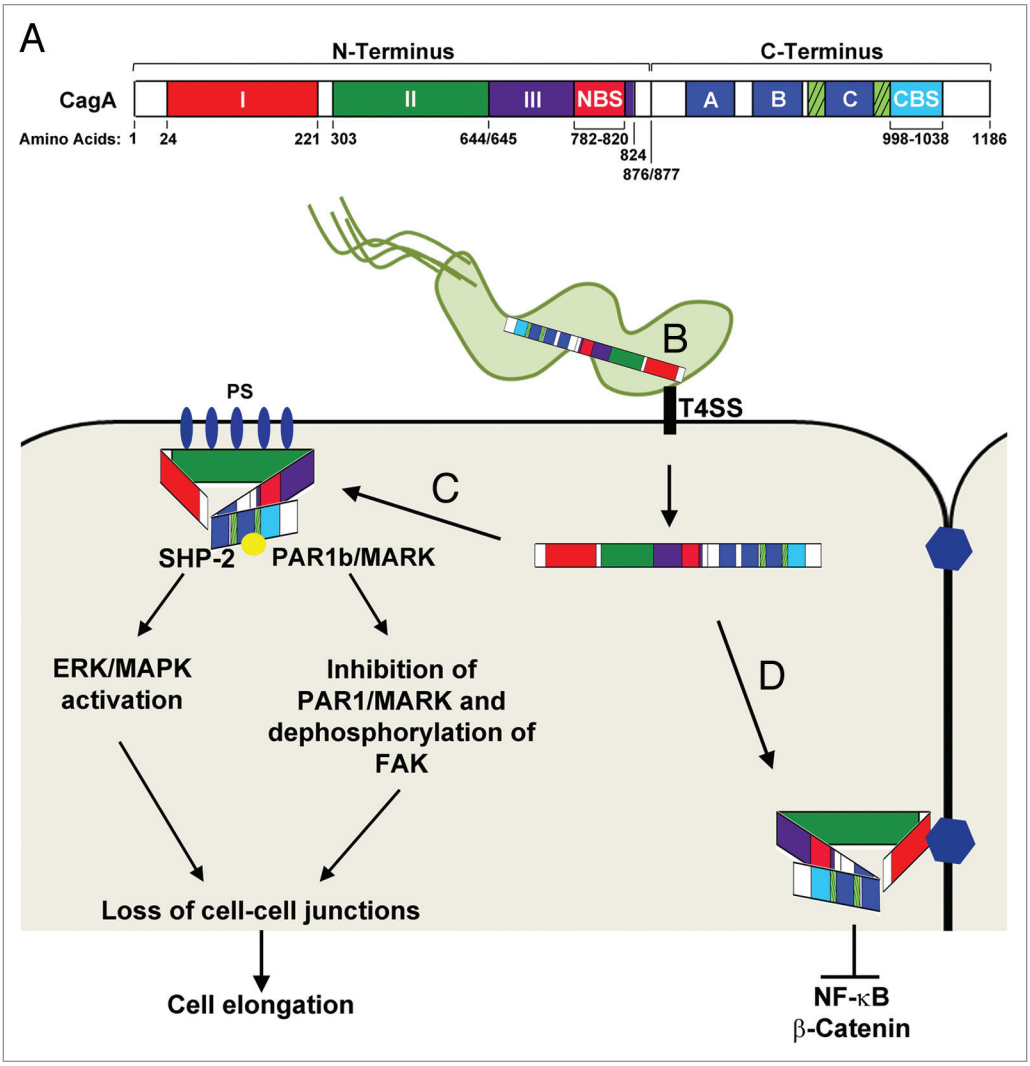

Figure 1. CagA functional domains. (**A**) Linear representation of CagA domains. The CagA N-terminus (1–876) contains three domains (I, II, and III). Domain I spans residues 24–221, Domain II spans residues 303–644, and Domain III spans residues 654–824. A N-terminal binding sequence (NBS) is located within Domain III from residues 782–820. The CagA C-terminus (877–1186) contains the EPIYA-ABC motifs (dark blue boxes), the Cag multimerization sequences (hatched boxes), and a C-terminal binding sequence (CBS) located from residues 998–1038.[90] (**B**) The first 200 amino acids of the N-terminus (Domain I) target CagA inside *H. pylori* to the type IV secretion system (T4SS). The T4SS interacts with β-1 integrin (black rectangle) and is responsible for translocation of CagA into the epithelial cell. Residues 800–1216 alone target CagA to the cytoplasm.[88] (**C**) CagA interacts with phosphatidylserine (PS; blue ovals) on the inner surface of the plasma membrane via basic residues located on Domain II. Interaction of the NBS and CBS condenses the structure of CagA allowing the unstructured C-terminus to remain accessible to Src-kinases as well as SHP-2. Phosphorylation of CagA (yellow circle) by Src-kinases results in activation of SHP-2 and the ERK/MAPK pathways leading to cell elongation. (**D**) Targeting of CagA to cell-cell junctions by Domain I results in inhibition of NF-κB and β-catenin and inhibition of EPIYA mediated cell signaling pathways. For more detailed information concerning the signaling pathways affected by CagA see reference 78. Figure was adapted from references 89, 90 and 92.

sequences that flank the EPIYA repeats. This variation has been used as a means to classify the EPIYA containing regions into motifs termed EPIYA-A, -B, -C, and -D. Furthermore, since geographic variability exists in grouping of these motifs, this has been used to classify CagA as either Western (contains EPIYA-A, -B, and -C, where the C motif may be duplicated multiple times) or East Asian (contains EPIYA-A, -B, and -D).[70,76,93,98] Recently the sequence and EPIYA motifs of two additional CagA motifs, J-Western and Amerindian, have also been characterized[10,20-22,24,25,99] and compared with the Western and East Asian CagA EPIYA motifs (**Fig. 2**).[23,100] Analysis of both nucleotide and amino acid sequences across *H. pylori* strains suggests that all CagA variants can be derived by homologous recombination at the CM sequence, recombination surrounding the EPIYA sequence, or by illegitimate recombination between short similar DNA sequences (1–12 base pairs) that appear to be AT rich.[101] Moreover, as discussed below, additional studies have investigated evolution of the J-Western and Amerindian motifs.

Analysis of Amerindian CagA EPIYA motifs show that the last EPIYA motif appears to be a hybrid between Western EPIYA-C and East Asian EPIYA-D motifs and thus, has been designated EPIYA-DC (**Fig. 2C**).[23] As such, the Amerindian

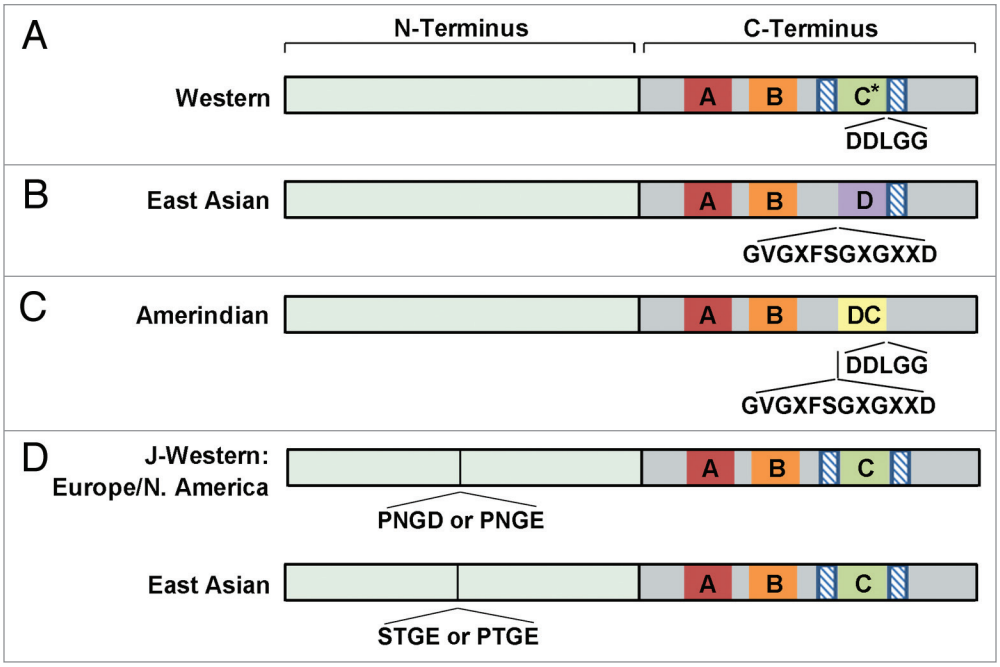

Figure 2. CagA variations by geographic region. (**A**) Western, (**B**) East Asian CagA, and D J-Western CagA contain a Cag multimerization (CM)/CRPIA/MKI sequence (FPL XRX XVX DLS KVG) for CagA dimerization and binding to PAR1/MARK (hatched boxes). *Western CagA can have up to five EPIYA-C repeats. (**C**) The last EPIYA motif of Amerindian CagA contains a motif that is a chimera of the East Asian EPIYA-D and Western EPIYA-C motifs as determined by the presence of a five amino acid sequence found in Western CagA (DDL GG) and a 12 amino acid sequence found in East Asian CagA (GVG XFS GXG XXD). (**D**) J-Western strains of either European or North American origin contain either a PNGD or PNGE N-terminal amino acid insertion, while strains of East Asian origin contain a STGE or a PTGE N-terminal amino acid insertion starting at residue 207 when compared with *H. pylori* strain 26695. Figure generated from studies described in reference 23.

CagA EPIYA-DC sequence contains a five amino acid sequence (DDLGG) found in the EPIYA-C motifs, and a 12 amino acid sequence (GVG XFS GXG XXD) that is similar to a segment found in EPIYA-D motifs.[23] Regardless of how Amerindian CagA evolved, the result is that Amerindian CagA appears less virulent than both Western and East Asian CagA; the level of IL-8 induction is decreased and it appears that Amerindian CagA is capable of serving as a dominant negative inhibitor of CagA.[25]

In comparison, initial analysis suggested that the EPIYA motifs of J-Western CagA were indistinguishable from Western CagA. However, further analysis of strains from Okinawa that also contain the *vacA* m2 allele (discussed below) and analysis of indels or hypervariable regions in the CagA proteins, reveal a four amino acid insertion in the N-terminus of J-Western CagA that is not present in any of the other forms of CagA (**Fig. 2D**).[23,100] In conjunction with the discovery that J-Western CagA has geographical origins other than Okinawa, Japan, it was found that this four amino acid sequence also varies based on *H. pylori* origin.[23] In this regard, a PNGD or PNGE insertion is present in J-Western CagA's that have a European or North American geographical origin, while a STGE or PTGE insertion is present in strains with an East Asian origin.[23] Furthermore, while 45 amino acid differences exist between J-Western and Western CagA, 112 differences exist between J-Western and East Asian CagA.[23]

Similar to variations in the C-terminal EPIYA motifs, analysis of the CagA N-terminus suggests that N-terminal recombination events may also have occurred; CagA from strains isolated in Japan show similarity to CagA from strains isolated from various locations around the world as well as similarity to East Asian CagA.[23] Therefore, Duncan et al. suggest that since the diversity is specific to parts of *cagA*, and is not present throughout the gene, these recombination events are coupled to selection.[23] Thus, the derivation of the various CagA alleles appears to involve a series of rearrangements/recombinations that occurred during the course of bacterial evolution within the host.

An immediate result of the variations in the EPIYA motifs can be seen by differences in tyrosine phosphorylation of the CagA EPIYA-ABC and EPIYA-ABD motifs. Elegant studies by Mueller et al. investigated the process of tyrosine phosphorylation of both the EPIYA-ABC and EPIYA-ABD motifs by Src family kinases.[102] These studies revealed that c-Src can phosphorylate both the EPIYA-C and -D motifs and that this phosphorylation occurs within 90 min of CagA translocation. Conversely, c-Abl can phosphorylate all four varieties of EPIYA motifs and this phosphorylation occurs within 180 min of translocation. Interestingly, the "hummingbird phenotype" cannot be induced by phosphorylation of any single motif in Western CagA, indicating that phosphorylation of at least two motifs is required for cell elongation.[102] Finally, the study demonstrated that single CagA molecules are simultaneously phosphorylated at only one or two EPIYA motifs, and never at all three motifs.[102] Since CagA is known to dimerize,[93] the dual phosphorylation may occur either on one CagA molecule or such that each monomer contains one phosphorylated EPIYA motif.[102] En masse, the model

that arises from these studies is that CagA is first phosphorylated by c-Src at the EPIYA-C or -D motif, followed by phosphorylation of the EPIYA-A or -B motif by c-Abl.[102] The fact that CagA can only be simultaneously phosphorylated at a maximum of two motifs, begs the question of why a substantial increase in apparent levels of phosphorylation are observed when cells are infected with an EPIYA-ABD motif containing strain as compared with an EPIYA-ABC motif containing strain.[93,103,104] One possibility could be that numerous components interplay to facilitate this finding. This notion is supported by previous studies showing that East Asian CagA forms a stronger interaction with SHP-2[80,104,105] due to the presence of a perfect SHP-2 consensus binding motif located around the EPIYA-D motif; in Western EPIYA-C motifs, the sequence differs at the pY+5 amino acid.[106] Once this high affinity interaction occurs, CagA dimerization mediated by the CM sequence through interaction with PAR1 dimers within the cell could facilitate a stronger interaction of CagA with SHP-2, resulting in increased SHP-2 deregulation and cell elongation.[107] In turn, phosphorylation of the EPIYA-A and -B motifs results in interaction of CagA with Csk, and Csk serves to inhibit Src functioning in a negative feedback loop.[105,108] In this model, Western CagA with an EPIYA-ABC motif binds more efficiently to Csk than an East Asian EPIYA-ABD motif, despite the ability of EPIYA-A and EPIYA-B motifs from both Western and East Asian CagA to bind Csk.[105] Therefore, it appears that this feedback loop is enhanced in Western strains.[105] Thus it is possible that the increase in binding affinity of EPIYA-D to SHP-2, CagA multimerization, and inhibition of Src activity by increased EPIYA-A and -B motifs in Western strains may be the reason underlying the apparent increase in phosphorylation observed with EPIYA-D motifs.

Role of the CagA EPIYA motifs in disease. Given the high degree of variability of the CagA EPIYA region, the major question that arises is whether this variability and subsequent differences in phosphorylation affect *H. pylori* mediated disease? This seems to be the case since studies of *H. pylori* strains isolated from patients in the United States, Italy, Brazil, and Portugal have shown that CagA containing higher numbers of EPIYA-C motifs are associated with the development of gastric carcinoma[109-112] or more severe precancerous lesions.[113] However, in direct contrast, study of *H. pylori* strains isolated from patients in Columbia, Venezuela, Mexico, and another Italian population showed no correlation between increasing EPIYA-C motifs and disease.[109,114-116] These differences in the association of the CagA EPIYA-C motifs and disease severity may be attributed to geographical differences in the EPIYA motifs themselves.[111,116] Conversely, the conflicting data may suggest that other factors must affect the ultimate role of CagA polymorphism on disease.

The idea that geographical diversity affects disease outcome is supported by molecular epidemiological studies of *H. pylori* strains that carry East Asian CagA. Early evaluation of Korean *H. pylori* strains did not find an association between disease and higher numbers of EPIYA-C motifs.[117] It should be noted that the findings of these studies are likely affected by sample size and the fact that East Asian strains typically carry the EPIYA-ABD motif. Indeed, work by Argent et al. found that 88.3% of East Asian strains harbored the EPIYA-ABD allele and these authors suggested that Western strains may in fact increase the number of EPIYA-C motifs as a means to increase virulence.[98] As such, co-culture studies of strains carrying various EPIYA motifs showed that East Asian strains expressing the EPIYA-ABD motif induced significantly more IL-8 secretion than Western strains with a single EPIYA-C motif; yet as the number of EPIYA-C motifs increased, the amount of IL-8 secreted also significantly increased.[98] Analysis of *H. pylori* strains from Japanese cancer patients provided the first evidence for the role of the EPIYA-ABD allele in *H. pylori* CagA associated carcinoma; all of the isolated strains carried the EPIYA-D motif.[104] Additionally, sera from Japanese patients was highly reactive to a 100 kDa fragment of CagA that was isolated from Japanese clinical isolates using a high-molecular weight cell-associated proteins (HM-CAP) assay.[118] In contrast, when this assay was performed using isolates from the United States, a larger CagA fragment that was not as reactive as the Japanese HM-CAP fragment was generated.[118] These different sized cleavage products suggest that there may also be structural differences in the various CagA proteins.[118] Of note, these proposed structural differences do not appear to alter the stability of CagA within the host cell since CagA EPIYA-ABD and CagA EPIYA-ABCCC do not exhibit a statistically significant difference in their half-lives.[83] Finally, the first definitive evidence for the role of the EPIYA-ABD motif in cancer development was obtained from a large scale molecular epidemiological study of South Korean *H. pylori* isolates; this work was the first to show that the presence of an EPIYA-ABD motif was statistically associated with the development of gastric cancer.[119] Taken together, current studies suggest that gastric cancer in East Asian countries is most often associated with the EPIYA-ABD motif[119] and more severe disease in Western countries is associated with multiple EPIYA-C motifs.[109-112]

CagA was first classified as an oncoprotein following studies using a C57BL/6J transgenic mouse model in which CagA containing an EPIYA-ABDD was expressed in the stomach of these animals.[120,121] This resulted in the formation of gastric polyps and adenocarcinoma of the stomach and small intestine; this was dependent on tyrosine phosphorylation of CagA and deregulation of SHP-2.[120] Notably, due to transgenic expression in this model, CagA EPIYA-ABDD also became systemically expressed and caused hematological abnormalities in some animals. Development of these conditions is attributed to the deregulation of SHP-2 by CagA EPIYA-ABDD,[120] which is known to avidly bind and activate SHP-2.[105] Interestingly, gastric tumor formation in the CagA EPIYA-ABDD transgenic mice occurs in the absence of overt mucosal inflammation and metaplastic change, suggesting that the formation of gastric carcinoma may not require chronic inflammation.[120] This is in contrast to the current belief that inflammation is crucial for the formation of precancerous lesions, which are believed to result from pathogenic T-cell mediated immune responses. Indeed, the absence of lymphocytes results in resistance to gastritis.[53,58] To study the role of the Western EPIYA motif in CagA induced carcinogenesis, a second transgenic mouse model was also developed.[121] Expression of CagA containing the EPIYA-ABCCC motif in these animals

induced thickening of the gastric mucosa comparable to that seen in the EPIYA-ABDD transgenic model.[121] Similar to the earlier study,[120] Western CagA induced tumor formation in the absence of chronic inflammation and intestinal metaplasia; however, the incidence of tumor formation was less than that induced by CagA EPIYA-ABDD and no hematological abnormalities developed.[121] Interestingly, both studies showed that once the CagA-induced transformed phenotype was established, CagA was no longer required for disease progression indicating that CagA plays a role in the early steps of gastric carcinoma.[120,121] The discrepancy that tumor formation was induced in the absence of inflammation, a condition previously suggested to be required for carcinogenesis in humans,[121] may be a side effect of the use of a transgene model. Together, these findings suggest that while Western CagA can induce tumor formation, it appears less potent than East Asian CagA. Given the differential effects on SHP-2, the authors suggest that tumor formation requires a threshold level of SHP-2 activation; the increase in SHP-2 activity induced by East Asian CagA may be the contributing factor to the increase in gastric carcinoma in East Asian countries.[121]

At the same time that the transgenic mouse model was developed, a CagA transgenic Drosophila model also emerged.[122] This model showed that expression of CagA results in apical targeting and epithelial disorganization and that CagA mimics the eukaryotic Grb2-associated binder (Gab) adaptor protein that can activate SHP-2.[122] However, later work showed that in this model, targeting of CagA to the apical region of the epithelium depends on EPIYA motif activation of myosin light chain (MLC) and not SHP-2.[123] Furthermore, work using a S2 cell culture model showed that CagA activates MLC in a Rho and Rho-Kinase (ROCK) dependent manner.[123] The role of Rho/ROCK in epithelial disruption is consistent with an earlier study conducted in AGS cells that shows that the characteristic "hummingbird phenotype" induced by CagA resulted from a failure of the trailing edge to retract, a process regulated by Rho and ROCK.[124] Those studies suggest that Rho and MLC, rather than SHP-2, may be the critical host cell proteins required for epithelial disruption.[123] Overall, the use of different cell culture and transgenic models has led to a dispute concerning exactly how CagA affects host cell signaling pathways. As such, it is clear that a common robust infection model is needed to tweeze out the mechanism underlying CagA carcinogenesis.

VacA

A second important toxin in *H. pylori*'s arsenal of virulence factors is VacA. *vacA* was discovered in 1988 and unlike *cagA*, which is not found in all strains, is present in virtually all *H. pylori* strains (refer to reviews in refs. 125 and 126). VacA is a pore forming toxin that is initially expressed as a 140 kDa protoxin and, following secretion via an autotransporter mechanism, is cleaved into a mature 88 kDa secreted toxin.[127] This secreted form is further cleaved to form 33 kDa and 55 kDa fragments termed p33 and p55, respectively (reviewed in refs. 126, 128 and 129). Though clearly not prototypical, VacA has been suggested to have structural similarity to AB toxins.[130] Moreover, recent discussion suggests that VacA could serve as a model for a new subtype of AB toxins, whereby the A subunit (comprised of p33) is responsible for pore formation rather than enzymatic activity, and the B subunit (composed of p55) is responsible for interaction with the host cell membrane.[128] In order for VacA to induce its toxic effects, it binds to sphingomyelin or the receptor type protein-tyrosine phosphatases RPTPα and RPTPβ, both of which are contained within eukaryotic cell membrane lipid rafts.[131-133] Following Cdc42 dependent pinocytic uptake, VacA is trafficked to the mitochondria where it induces apoptosis and formation of large intracellular vacuoles.[127,134] While the mechanism underlying VacA targeting to the mitochondria remains unclear, it is clear that VacA induced pore formation in the mitochondrial membrane is responsible for induction of apoptosis (reviewed in refs. 126, 129 and 135).

As a vast majority of information is known about how VacA functions and signals in host cells (refer to reviews in refs. 78, 126, 129, 135 and 136), herein, we will only briefly mention some discoveries that affect our understanding of how VacA polymorphisms influence disease state. Many molecular epidemiological studies suggest that VacA is important for disease (discussed below). Furthermore, analysis of sera from patients with intestinal metaplasia, gastric carcinoma, duodenal ulcers, and non-atrophic gastritis suggests an association between neutralizing VacA antibodies and gastric carcinoma; the amount of neutralizing antibodies tended to increase as patients progressed from pre-neoplastic lesions to those with cancer.[137] Additionally, serum-neutralizing antibodies appear to depend on *vacA* polymorphism (discussed below), and antibodies to VacA also appear to persist longer than antibodies to *H. pylori* itself; in patients who tested negative for *H. pylori* but positive for VacA, the risk for gastric carcinoma was increased compared with those who tested positive for both.[137]

VacA polymorphisms. Similar to *cagA*, *vacA* also contains allelic diversity that varies across *H. pylori* strains.[138] VacA contains four identified variable regions, each of which are subdivided into types (**Fig. 3**). The best characterized regions are the signal (s) region, which encompasses the N-terminus and a portion of the signal sequence and is typed as s1 or s2; and the mid (m) region of the gene, which is typed as m1 or m2.[138] Since they were only recently discovered, much less is known about the role of the intermediate (i) region or the deletion (d) region in disease progression.[139,140] The i-region lies between the s- and m-regions and is typed as i1, i2, or i3 (**Fig. 3**).[139,141] The d-region, located between the i- and m-regions is typed as d1 if there is no deletion (**Fig. 3A**), or d2 if a 69 to 89 base pair deletion is present (**Fig. 3B**).[140]

vacA can recombine to form a wide variety of combinations, some of which are less common than others. Of the possible allelic combinations, the *vacA* s1/m1 alleles are the most virulent combination, while the s1/m2 and s2/m2 genotypes display virtually no cytotoxicity.[138,142-147] Subsequent identification of the i-region showed that the i1 polymorphism is a significant, independent risk factor for gastric carcinoma[139] and is strongly associated with the s1 polymorphism, both of which are associated with peptic ulcers.[148] As such, it has been suggested that typing the i-region

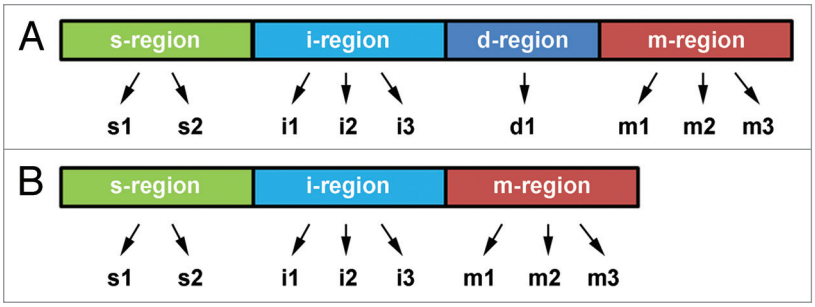

Figure 3. *vacA* polymorphism. Like *cagA*, *vacA* contains polymorphism that results in VacA toxins with varying degrees of virulence. Each region of the gene has multiple alleles (indicated by arrows), which can combine in any combination. (**A**) While the s-, i-, and m-regions are always present, the presence of the d-region is termed d1. (**B**) VacA that contains a 69–89 base pair deletion between the i- and m-regions is termed d2. Figure was modified from reference 135.

of VacA may suffice to determine the pathogenic potential of the toxin.[139,148] While only one study has investigated the role of the d-region in disease, this study showed that the s1, m1, i1, and d1 polymorphisms significantly increase the risk for gastric cancer in Western countries.[140] Based on the results of that and a previous study by the same group, it was suggested that the d-region polymorphism could be used as a better predictor for disease.[140] Together, while each of the *vacA* polymorphisms has been used as a predictor for VacA-induced disease severity, it is clear that other factors, such as the presence of CagA (discussed below), contribute to disease. For this reason individually typing *vacA* alone may not provide sufficient information to understand the virulence potential of a strain and typing of multiple virulence factors appears to be required to understand strain dependent disease contributions.

Epidemiological Connection between CagA and VacA

Since the mid-1990s, studies have attempted to correlate the independent presence of CagA, VacA, and several other virulence factors with disease state, oftentimes producing mixed results (discussed below). Studies conducted in Senegal, Taiwan, South Korea, Greece, Turkey, Alaska, and Europe identified a correlation between CagA status and more severe disease states (duodenal ulcers, peptic ulcer disease, progression of preneoplastic lesions, and gastric cancer).[42,144,149-156] Additionally, patients identified as VacA seropositive were shown to have an increased risk of gastric carcinoma that occurred in conjunction with the s1/m1 polymorphism.[157-159] It has become clear that looking at these toxins independently is insufficient. Thus, examination of the combined *cagA* and *vacA* status of a strain has shown a significant association between the presence of both genes and disease state.[144,148,151-154,160] These studies show that most *cagA* positive *H. pylori* strains are associated with the *vacA* s1 and/or m1 genotypes.[42,99,144,150-155,161-164] Together these studies support the notion that individuals who carry *cagA* positive, *vacA* s1/m1 *H. pylori* isolates may be candidates for eradication therapy as a means to prevent severe disease (duodenal ulcers, peptic ulcer disease, progression of preneoplastic lesions, and gastric cancer).

Despite the evidence that *cagA* positivity, *cagA* and *vacA* seropositivity, and/or *vacA* polymorphism contribute to disease severity, numerous studies have not found this association.[163-174] For example, in Tunisia, *vacA* type is significantly different between patients with peptic ulceration and gastritis, while *cagA* status is not.[175] In China, no association between *cagA* status and peptic ulceration or chronic gastritis was established, likely due to the high presence of *cagA* in both patient populations.[164] The differences observed between disease severity and toxin type/presence in these epidemiological studies may be due to differences that exist in *H. pylori* strains well beyond the described *cagA* and *vacA* polymorphisms. As noted at the outset of this review, environmental, geographic, and host influences could contribute to the differences observed in disease severity between these studies. As such, while individual evaluation of *cagA* and *vacA* genotypes show that both contribute to disease; the lack of evaluation of both genotypes in combination with other factors is problematic for determining how both toxins contribute to disease.[149]

Functional Interaction between CagA and VacA

While numerous epidemiological studies have been conducted to determine how VacA and CagA are distributed between populations, the area that clearly remains understudied is the functional connection between the two toxins (this topic has also recently been reviewed in ref. 129). The first study investigating the interaction between CagA and VacA found that CagA contains an amino acid sequence that resembles the immune-receptor tyrosine based activation motif (ITAM) that is found on the cytoplasmic region of immune receptors; tyrosine phosphorylated CagA localizes to and clusters in lipid rafts.[132] Furthermore, CagA can associate with receptor tyrosine phosphorylated GIT1/Cat1, which is a substrate of the VacA receptor RPTPα/β.[132,133] The authors suggested that the interaction between the toxins in lipid rafts results in negative regulation; VacA appears responsible for reduced tyrosine phosphorylation of CagA during early stages of infection. A second study illustrated another CagA and VacA functional interaction. This study showed that CagA activates the PLC-γ-Ca^{2+}-calcineurin pathway, which results in translocation of NFAT to the nucleus and transcription of NFAT regulated genes.[176] To counteract CagA-induced effects on host cell signaling, VacA inhibits T-cell Ca^{2+} uptake thus preventing dephosphorylation of calcineurin and translocation of NFAT to the nucleus.[176]

While CagA and VacA can prevent host cell morphological changes induced by the other, it remains unclear how this process occurs.[177] A study investigating host cell signaling pathways showed that VacA prevents CagA-induced cell elongation by suppressing the EGRF and HER2/Neu receptors, resulting in inhibition of the Erk1/2 kinase pathway.[178] To prevent VacA-induced

host cell apoptosis, phosphorylated CagA decreases SKF tyrosine activity within the host cell and blocks trafficking of VacA containing compartments the mitochondria; CagA does not appear to inhibit pinocytotic uptake of VacA.[179] Therefore, it was suggested that for the small amount of VacA that reaches the mitochondria, non-phosphorylated CagA may induce nuclear translocation of NF-κB and production of IL-8 as a means to block VacA induced apoptosis.[179] In direct contrast, a later study showed that in host cells with attached *H. pylori*, translocated CagA inhibits VacA-induced apoptosis by preventing pinocytotic uptake of VacA, resulting in a decrease in vacuole formation within the host cell.[180] Moreover, uninfected cells were susceptible to VacA induced apoptosis.[180] Finally in terms of CagA and VacA interaction, a recent study revealed that *H. pylori* may utilize both CagA and VacA for acquisition of iron from mucosal epithelial cells.[181] In this model, CagA increases transferrin uptake from the basolateral surface in an EPIYA dependent manner, delivering it to the apical surface to be released into the lumen, while VacA increases transferrin-receptor uptake from the basolateral surface, delivering it to sites of bacterial attachment.[181] Akada et al. suggest that when *H. pylori* attach to gastric epithelial cells, CagA and VacA may interact in a manner that facilitates persistent colonization since CagA prevents VacA-mediated apoptosis. In contrast, when *H. pylori* are planktonic, VacA induces cellular apoptosis in both infiltrating immune cells and epithelial cells to acquire nutrients required for *H. pylori* survival.[180]

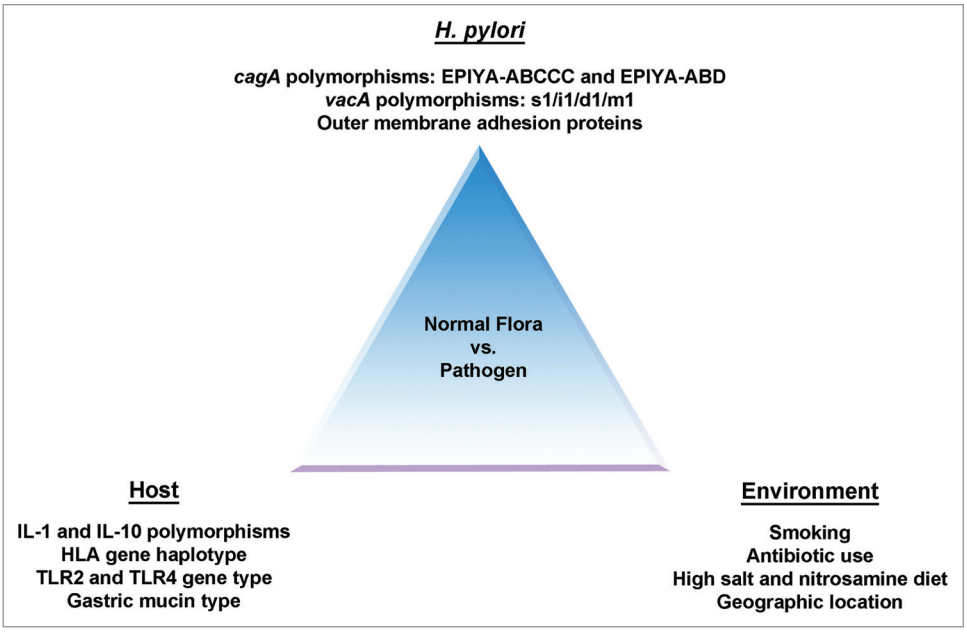

Figure 4. Balance between *H. pylori* as normal flora vs. a pathogen. The balance between *H. pylori*'s role in protecting against disease vs. causing gastric pathologies depends on bacterial, host and environmental factors. As such, based on the combination of these factors in any one individual, it may be detrimental to eradicate *H. pylori* from the human stomach.

While these studies opened the door to understanding how CagA and VacA functionally interact and outline a direction for future studies, currently few studies have actually investigated in detail the combined role of CagA and VacA polymorphism in relation to disease state. One such study performed sequence analysis of the VacA i-region of *H. pylori* isolated from South Korean patients.[182] In this study, polymorphism at amino acid 196 is associated with more severe disease, and polymorphism at amino acid 231 is linked to strains that do not carry the CagA EPIYA-ABD allele. Together, the data suggest that polymorphisms within each toxin may affect the functional interaction between the two toxins.[182] Clearly, more intense study is required in this area.

Conclusion

Current evidence supports the hypothesis that *H. pylori* was acquired by humans prior to the great human migration out of Africa. As such, *H. pylori* has evolved mechanisms to persist within the harsh human gastric environment and may even serve to protect humans from diseases such as asthma, allergy, and esophageal cancer. While half of the world's population is infected with *H. pylori*, only a subset of individuals actually develop *H. pylori* induced disease. As such, a debate exists as to whether *H. pylori* should be eradicated from the human microbiome. Since recent studies suggest that numerous factors (**Fig. 4**) contribute to the establishment of disease, it perhaps seems more appropriate to individually assess each infected individual for eradication therapy. As the evidence is starting to suggest that *H. pylori* protects us against several disease states, overtly eradicating *H. pylori* from the human microbiome may open "Pandora's box" and be more detrimental than helpful to overall human health. However, if we can understand the mechanisms underlying the conversion of *H. pylori* from the commensal found in most of the world's population, to the pathogen only found in a subset of individuals, we would have the means to selectively treat those who are at the greatest risk for *H. pylori* induced gastric carcinoma. As such, there are several questions regarding the pathogenic mechanism of *H. pylori* that require further study: (1) How do the different CagA EPIYA motifs affect host cell signaling pathways; (2) Does CagA inhibit VacA induced apoptosis at the step of pinocytosis or does CagA target a protein required for vesicular transport within the host to inhibit VacA trafficking; (3) How do the polymorphisms in each toxin affect the ability of CagA and VacA to functionally interact; and (4) How does this interaction ultimately contribute to disease severity? Future studies will aim to answer these questions in order to provide a better understanding of *H. pylori*-induced gastric disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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